WHAT IS CLAIMED IS:

1. A method for evolving a protein encoded by a DNA substrate molecule comprising:

(a) digesting at least a first and second DNA substrate molecule, wherein the at least a first and second substrate molecules differ from each other in at least one nucleotide, with a restriction endonuclease;

(b) ligating the mixture to generate a library of recombinant DNA molecules;

(c) screening or selecting the products of (b) for a desired property; and

(d) recovering a recombinant DNA substrate molecule encoding an evolved protein.

2. The method of claim 1, wherein the restriction endonuclease generates non-palindromic ends at cleavage sites.

3. The method of claim 1, wherein the substrate molecules have been engineered to contain at least one recognition site for a restriction endonuclease having non-palindromic ends at cleavage sites.

4. The method of claim 1, wherein (a) - (d) are repeated.

5. The method of claim 1, wherein the DNA substrate molecule comprises a gene cluster.

6. The method of claim 1, wherein at least one restriction endonuclease fragment from a DNA substrate molecule is isolated and subjected to mutagenesis to generate a library of mutant fragments.

7. The method of step 6, wherein the library of mutant fragments is used in the ligation of (b).

8. The method of claim 7, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

9. The method of claim 6, wherein mutagenesis comprises recursive sequence recombination.

10. The method of claim 1, wherein the products of (d) are subjected to mutagenesis.

11. The method of claim 10, wherein mutagenesis comprises recursive sequence recombination.

12. The method of claim 1, wherein the products of (d) are used as a DNA substrate molecule in (b).

13. The method of claim 10, wherein the products of claim 10 are used in (d).

14. The method of claim 1, wherein the recombinant DNA substrate molecule of (d) comprises a library of recombinant DNA substrate molecules.

15. An evolved protein produced by the method of claim 1.

16. A method for evolving a protein encoded by a DNA substrate molecule by recombining at least a first and second DNA substrate molecule, wherein the at least a first and second substrate molecules differ from each other in at least one nucleotide and comprise defined segments, the method comprising:

(a) providing a set of oligonucleotide PCR primers, comprising at least one primer for each strand of each segment, wherein the primer sequence is complementary to at least one junction with another segment;

(b) amplifying the segments of the at least a first and second DNA substrate molecules with the primers of step (a) in a polymerase chain reaction;

(c) assembling the products of step (b) to generate a library of recombinant DNA substrate molecules;

(d) screening or selecting the products of (c) for a desired property; and

(e) recovering a recombinant DNA substrate molecule from (d) encoding an evolved protein.

17. The method of claim 16, wherein the at least a first and second DNA substrate molecules are subjected to mutagenesis prior to step (a).

18. The method of claim 16, wherein the at least a first and second DNA substrate molecules comprise alleles of a gene.

19. The method of claim 16, wherein the at least a first and second DNA substrate molecules comprise a library of mutants.

20. The method of claim 16, wherein the segments are defined by sites within intergenic regions.

21. The method of claim 16, wherein the segments are defined by sites within introns.

22. The method of claim 16, wherein the primers comprise a uracil substitution at one or more thymidine residues.

23. The method of claim 22, wherein the products of (b) are treated with uracil glycosylase.

24. The method of claim 16, wherein (a) - (e) are repeated.

25. The method of claim 16, wherein the at least a first and second DNA substrate molecule comprises a gene cluster.

26. The method of claim 16, wherein the at least first and second DNA substrate molecule encodes all or part of a DNA polymerase.

27. The method of claim 16, wherein at least one PCR primer differs from the at least a first and second DNA substrate molecules in at least one nucleotide.

28. The method of claim 27, wherein the PCR primer comprises a nucleotide sequence of a known mutant or polymorphism of the at least a first or second DNA substrate molecule.

29. The method of claim 28, wherein the PCR primer is degenerate and encodes the nucleotide sequences of more than one known mutant or polymorphism of the at least a first or second DNA substrate molecule.

30. The method of claim 29, wherein the at least a first and second DNA substrate molecule encodes all or part of a protein selected from Table I.

31. The method of claim 17, wherein mutagenesis comprises recursive sequence recombination.

32. The method of claim 16, wherein the products of (e) are subjected to mutagenesis.

33. The method of claim 32, wherein mutagenesis comprises recursive sequence recombination.

34. The method of claim 32, wherein the products of claim 32 are used in (b).

35. The method of claim 16, wherein the products of (e) are used as a DNA substrate molecule in (b).

36. The method of claim 16, wherein the recombinant DNA

substrate molecule of (e) comprises a library of recombinant DNA substrate molecules.

37. An evolved protein produced by the method of claim 16.

38. A method of enriching a population of DNA fragments for mutant sequences comprising:

(a) denaturing and renaturing the population of fragments to generate a population of hybrid double-stranded fragments in which at least one double-stranded fragment comprises at least one base pair mismatch;

(b) fragmenting the products of (a) into fragments of about 20-100 bp;

(c) affinity-purifying fragments having a mismatch on an affinity matrix to generate a pool of DNA fragments enriched for mutant sequences; and

(d) assembling the products of (c) to generate a library of recombinant DNA substrate molecules.

39. The method of claim 38, wherein the population of DNA fragments is derived from at least a first and second DNA substrate molecule, the at least a first and second DNA substrate molecule differing from each other in at least one nucleotide.

40. The method of claim 39, wherein the at least a first and second DNA substrate molecules are obtained by mutagenesis of a DNA substrate molecule.

41. The method of claim 39, wherein the at least a first and second DNA substrate molecules comprise alleles of a gene.

42. The method of claim 39, wherein the at least a first and second DNA substrate molecules comprise polymorphic variants of a gene.

43. The method of claim 38, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

44. The method of claim 38, wherein the products of (c) are mixed with the products of (a) prior to (d).

45. A method for evolving a protein encoded by a DNA substrate molecule, by recombining at least a first and second DNA substrate molecule, wherein the at least a first and second substrate molecules share a region of sequence homology of about 10 to 100 base pairs and comprise defined segments, the method comprising:

(a) providing regions of homology in the at least a first and second DNA substrate molecules by inserting an intron sequence between at least two defined segments;

(b) fragmenting and recombining DNA substrate molecules of (a), wherein regions of homology are provided by the introns;

(c) screening or selecting the products of (b) for a desired property; and

(d) recovering a recombinant DNA substrate molecule from the products of (c) encoding an evolved protein.

46. The method of claim 45, wherein the introns are self-splicing.

47. The method of claim 45, wherein the inserted introns comprise from about 1 to about 10 nonhomologous introns.

48. The method of claim 45, wherein the intron comprises a recognition site for a restriction endonucleases having non-palindromic ends at cleavage sites.

49. The method of claim 45, wherein (b) - (d) are repeated.

50. The method of claim 45, wherein the DNA substrate molecule comprises a gene cluster.

51. The method of claim 45, wherein at least one segment from a DNA substrate molecule is isolated and subjected to mutagenesis to generate a library of mutant fragments.

52. The method of claim 51, wherein the library of mutant segments is used in the recombination of (b).

53. The method of claim 45, wherein the segments are defined by exons.

54. The method of claim 45, wherein the segments are defined by intergenic regions.

55. The method of claim 45, wherein the at least a first and second DNA substrate molecules encode protein homologues.

56. The method of claim 45, wherein the intron contains a lox site, and wherein the products of (b) are used to transfect a Cre^+ host.

57. The method of claim 45, wherein the at least a first and second DNA substrate molecule encodes all or part of a protein selected from Table I.

58. The method of claim 45, wherein the at least a first and second DNA substrate molecule are subjected to mutagenesis prior to step (a).

59. The method of claim 58, wherein mutagenesis comprises recursive sequence recombination.

60. The method of claim 45, wherein the products of (d) are subjected to mutagenesis.

61. The method of claim 58, wherein mutagenesis comprises recursive sequence recombination.

62. The method of claim 45, wherein the products of (d) are used as a DNA substrate molecule in (b).

63. The method of claim 45, wherein the recombinant DNA substrate molecule of (d) comprises a library of recombinant DNA substrate molecules.

64. An evolved protein produced by the method of claim 45.

65. A method for evolving a protein encoded by a DNA substrate molecule by recombining at least a first and second DNA substrate molecule, wherein the at least a first and second substrate molecules differ from each other in at least one nucleotide and comprise defined segments, the method comprising:

(a) providing a set of oligonucleotide PCR primers, wherein for each junction of segments a pair of primers is provided, one member of each pair bridging the junction at one end of a segment and the other bridging the junction at the other end of the segment, with the terminal ends of the DNA molecule having as one member of the pair a generic primer, and wherein a set of primers is provided for each of the at least a first and second substrate molecules;

(b) amplifying the segments of the at least a first and second DNA substrate molecules with the primers of (a) in a polymerase chain reaction;

(c) assembling the products of (b) to generate a pool of recombinant DNA molecules;

(d) selecting or screening the products of (c) for a desired property; and

(e) recovering a recombinant DNA substrate molecule from the products of (d) encoding an evolved protein.

66. The method of claim 65, wherein (a) - (e) is repeated.

67. The method of claim 65, wherein the at least a

first and second DNA substrate molecule are subjected to mutagenesis prior to (a).

68. The method of claim 65, wherein the at least a first and second DNA substrate molecule comprise sequences encoding protein homologues.

69. The method of claim 65, wherein the primers comprise a uracil substitution at one or more thymidine residues.

70. The method of claim 69, wherein the products of (b) are treated with uracil glycosylase.

71. The method of claim 65, wherein the at least a first and second DNA substrate molecule encodes all or part of a protein selected from Table I.

72. The method of claim 65, wherein the at least a first and second DNA substrate molecule comprises a gene cluster.

73. An evolved protein produced by the method of claim 65.

74. The method of claim 65, wherein at least one PCR primer differs from the at least a first and second substrate molecules in at least one nucleotide.

75. The method of claim 74, wherein the PCR primer comprises a nucleotide sequence of a known mutant or polymorphism of the at least a first or second substrate molecule.

76. The method of claim 75, wherein the PCR primer is degenerate and encodes the nucleotide sequences of more than one known mutant or polymorphism of the at least a first or second substrate molecule.

77. The method of claim 67, wherein mutagenesis

comprises recursive sequence recombination.

78. The method of claim 65, wherein the products of (e) are subjected to mutagenesis.

79. The method of claim 78, wherein mutagenesis comprises recursive sequence recombination.

80. The method of claim 65, wherein the products of (e) are used as a DNA substrate molecule in (b).

81. The method of claim 65, wherein the recombinant DNA substrate molecule of (e) comprises a library of recombinant DNA substrate molecules.

82. A method for optimizing expression of a protein by evolving the protein, wherein the protein is encoded by a DNA substrate molecule, comprising:

(a) providing a set of oligonucleotides, wherein each oligonucleotide comprises at least two regions complementary to the DNA molecule and at least one degenerate region, each degenerate region encoding a region of an amino acid sequence of the protein;

(b) assembling the set of oligonucleotides into a library of full length genes;

(c) expressing the products of (b) in a host cell;

(d) screening the products of (c) for improved expression of the protein; and

(e) recovering a recombinant DNA substrate molecule encoding an evolved protein from (d).

83. The method of claim 82, wherein the primers comprise about 20 nucleotides complementary to the DNA substrate molecule followed by a second region of about 20 degenerate nucleotides of homology with the DNA substrate molecules followed by about 20 nucleotides complementary to the DNA substrate.

84. The method of claim 82, wherein the protein is bovine intestinal alkaline phosphatase.

85. The method of claim 84, wherein the oligonucleotides comprise one or more primers from Table II.

86. The method of claim 82, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

87. The method of claim 82, wherein the DNA molecule comprises a gene cluster.

88. The method of claim 82, wherein (a) - (e) are repeated.

89. The method of claim 82, wherein the oligonucleotides comprise at least 5' and 3' nucleotide complementary to the DNA substrate molecule and about 20-300 nucleotides having up to about 85% sequence homology with a region of the DNA substrate molecule.

90. The method of claim 89, wherein the oligonucleotides comprise a set of oligonucleotides in which each oligonucleotide overlaps with a second oligonucleotide.

91. The method of claim 82, wherein the products of (e) are subjected to mutagenesis.

92. The method of claim 91, wherein mutagenesis comprises recursive sequence recombination.

93. The method of claim 82, wherein the recombinant DNA substrate molecule of (e) comprises a library of recombinant DNA substrate molecules.

94. An evolved protein produced by the method of claim 82.

95. A method for optimizing expression of a protein encoded by a DNA substrate molecule by evolving the protein, wherein the DNA substrate molecule comprises at least one lac operator and a fusion of a DNA sequence encoding the protein with a DNA sequence encoding a lac headpiece dimer, the method comprising:

(a) transforming a host cell with a library of mutagenized DNA substrate molecules;

(b) inducing expression of the protein encoded by the library of (a);

(c) preparing an extract of the product of (b);

(d) fractionating insoluble protein from complexes of soluble protein and DNA; and

(e) recovering a DNA substrate molecule encoding an evolved protein from (d).

96. The method of claim 95, wherein (a) - (e) are repeated.

97. The method of claim 95, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

98. An evolved protein produced by the method of claim 95.

99. The method of claim 95, wherein the products of (e) are subjected to mutagenesis.

100. The method of claim 99, wherein mutagenesis comprises recursive sequence recombination.

101. The method of claim 95, wherein the products of (e) are used as a DNA substrate molecule in (a).

102. The method of claim 95, wherein the recombinant DNA substrate molecule of (e) comprises a library of recombinant DNA substrate molecules.

103. A method for evolving functional expression of a protein encoded by a DNA substrate molecule comprising a fusion of a DNA sequence encoding the protein with a DNA sequence encoding filamentous phage protein to generate a fusion protein, the method comprising:

(a) providing a host cell producing infectious particles expressing a fusion protein encoded by a library of mutagenized DNA substrate molecules;

(b) recovering from (a) infectious particles displaying the fusion protein;

(c) affinity purifying particles displaying the mutant protein using a ligand for the protein; and

(d) recovering a DNA substrate molecule encoding an evolved protein from affinity purified particles of (c).

104. The method of claim 103, wherein (a) - (d) are repeated.

105. The method of claim 103, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

106. An evolved protein produced by the method of claim 103.

107. The method of claim 103, wherein the products of (d) are subjected to mutagenesis.

108. The method of claim 107, wherein mutagenesis comprises recursive sequence recombination.

109. The method of claim 107, wherein the products of claim 107 are used as a DNA substrate molecule in (a).

110. The method of claim 103, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

111. The method of claim 103, wherein DNA sequence

encoding the filamentous phage protein comprises a phagemid.

112. The method of claim 103, wherein DNA sequence encoding the filamentous phage protein comprises a phage.

113. A method for optimizing expression of a protein encoded by a DNA substrate molecule comprising a fusion of a DNA sequence encoding the protein with a DNA substrate encoding a lac headpiece dimer, wherein the DNA substrate molecule is present on a first plasmid vector, the method comprising:

(a) providing a host cell transformed with the first vector and a second vector comprising a library of mutants of at least one chaperonin geneand at least one lac operator;

(b) preparing an extract of the product of (a);

(c) fractionating insoluble protein from complexes of soluble protein and DNA; and

(d) recovering DNA encoding a chaperonin gene from (c).

114. The method of claim 113, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

115. The method of claim 113, wherein the DNA substrate is subjected to mutagenesis independently of the chaperonin gene prior to (a).

116. The method of claim 113, wherein the DNA of (d) comprises a library of mutants.

117. The method of claim 113, wherein the first and second vectors are the same vector.

118. The method of claim 113, wherein (d) further comprises recovering an evolved DNA substrate molecule from the products of (c).

119. An evolved chaperonin produced by the method of claim 113.

120. An evolved protein produced by the method of claim 113.

121. The method of claim 113, wherein (a) - (d) are repeated.

122. The method of claim 113, wherein the products of (d) are subjected to mutagenesis.

123. The method of claim 122, wherein mutagenesis comprises recursive sequence recombination.

124. The method of claim 122, wherein the products of claim 122 are used in (a).

125. A method for optimizing expression of a protein encoded by a DNA substrate molecule comprising a fusion of a DNA sequence encoding the protein with a filamentous phage gene, wherein the fusion is carried on a phagemid comprising a library of chaperonin gene mutants, the method comprising:

(a) providing a host cell producing infectious particles expressing a fusion protein encoded by a library of mutagenized DNA substrate molecules;

(b) recovering from (a) infectious particles displaying the fusion protein;

(c) affinity purifying particles displaying the protein using a ligand for the protein; and

(d) recovering DNA encoding the mutant chaperonin from affinity purified particles of (c).

126. The method of claim 125, wherein (a) - (d) are repeated.

127. The method of claim 125, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

128. An evolved chaperonin produced by the method of

claim 125.

129. An evolved protein produced by the method of claim 125.

130. The method of claim 125, wherein the products of (d) are subjected to mutagenesis.

131. The method of claim 130, wherein mutagenesis comprises recursive sequence recombination.

132. The method of claim 130, wherein the products of claim 130 are used in (a).

133. The method of claim 125, wherein the DNA of (d) comprises a library of DNA substrate molecules.

134. The method of claim 125, wherein the DNA substrate molecule comprises a library of mutagenized DNA sequences encoding the protein of interest.

135. The method of claim 125, wherein (d) further comprises recovering DNA encoding the protein from affinity purified particles of (c).

136. A method for optimizing secretion of a protein in a host by evolving a gene encoding a secretory function, comprising:

(a) providing a cluster of genes encoding secretory functions;

(b) recombining at least a first and second sequence in the gene cluster of (a) encoding a secretory function, the at least a first and second sequences differing from each other in at least one nucleotide, to generate a library of recombinant sequences;

(c) transforming a host cell culture with the products of (b), wherein the host cell comprises a DNA sequence encoding the protein;

(d) subjecting the product of (c) to screening or selection for secretion of the protein; and

(e) recovering DNA encoding an evolved gene encoding a secretory function from the product of (d).

137. The method of claim 136, wherein the gene cluster comprises at least one recognition site for a restriction endonuclease having nonpalindromic ends at the cleavage site.

138. The method of claim 136, wherein the host is *E. coli.*, yeast, *Bacillus*, *Pseudomonas*, or a mammalian cell.

139. The method of claim 136, wherein the protein is a thermostable DNA polymerase.

140. The method of claim 136, wherein protein is inducibly expressed.

141. The method of claim 136, wherein the protein is linked to a secretory leader sequence.

142. A secretory gene evolved by the method of claim 136.

143. The method of claim 136, wherein (a) - (e) are repeated.

144. The method of claim 136, wherein the DNA sequence of (c) encodes all or part of a protein selected from Table I.

145. The method of claim 136, wherein the DNA sequence of (c) comprises a library of mutant sequences.

146. The method of claim 136, wherein the products of (e) are subjected to mutagenesis.

147. The method of claim 146, wherein mutagenesis

comprises recursive sequence recombination.

148. The method of claim 146, wherein the products of claim 146 are used in (a).

149. The method of claim 136, wherein the DNA of (e) comprises a library of evolved genes.

150. A method for evolving an improved DNA polymerase comprising:

(a) providing a library of mutant DNA substrate molecules encoding mutant DNA polymerase;

(b) screening extracts of cells transfected with (a) and comparing activity with wild type DNA polymerase;

(c) recovering mutant DNA substrate molecules from cells in (b) expressing mutant DNA polymerase having improved activity over wild-type DNA polymerase; and

(d) recovering a DNA substrate molecule encoding an evolved polymerase from the products of (c).

151. The method of claim 150, wherein the improved activity is at least one of the group of higher quality sequencing ladder, less termination of reactions with inosine, improve acceptance of base analogs, improved acceptance of dideoxy nucleotides, and longer sequencing ladders.

152. The method of claim 150, wherein the products of (a) are expressed under control of arabinose promoter in an E. coli host having a mutant host DNA polymerase.

153. The method of claim 150, wherein (a) - (d) are repeated.

154. An evolved DNA polymerase produced by the method of claim 150.

155. The method of claim 150, wherein the products of

(d) are subjected to mutagenesis.

156. The method of claim 155, wherein mutagenesis comprises recursive sequence recombination.

157. The method of claim 155, wherein the products of claim 155 are used in (a).

158. The method of claim 150, wherein the DNA substrate molecule of (d) comprises a library of DNA substrate molecules.

159. A method for evolving a DNA polymerase with an error rate greater than that of wild type DNA polymerase comprising:

(a) providing a library of mutant DNA substrate molecules encoding mutant DNA polymerase in a host cell comprising an indicator gene having a revertible mutation, wherein the indicator gene is replicated by the mutant DNA polymerase;

(b) screening the products of (a) for revertants of the indicator gene;

(c) recovering mutant DNA substrate molecules from revertants; and

(d) recovering a DNA substrate molecule encoding an evolved polymerase from the products of (c).

160. The method of claim 159, wherein the indicator gene is LacZalpha or GFP.

161. The method of claim 159 wherein the revertible mutation is a stop codon.

162. The method of claim 159, wherein the host cell comprises a mutant host DNA polymerase.

163. A method for evolving a DNA polymerase, comprising:

(a) providing a library of mutant DNA substrate molecules encoding mutant DNA polymerase, the library comprising a

plasmid vector;

(b) preparing plasmid preparations and extracts of host cells transfected with the products of (a);

(c) amplifying each plasmid preparation in a PCR reaction using the mutant polymerase encoded by that plasmid, the polymerase being present in the host cell extract;

(d) recovering the PCR products of (c); and

(e) recovering a DNA substrate molecule encoding an evolved polymerase from the products of (d).

164. The method of claim 163, wherein the reaction of (c) is carried out in the presence of an organic solvent, a base analog, or inosine.

165. The method of claim 163, wherein (a) - (e) are repeated.

166. An evolved polymerase produced by the method of claim 163.

167. The method of claim 163, wherein the products of (e) are subjected to mutagenesis.

168. The method of claim 167, wherein mutagenesis comprises recursive sequence recombination.

169. The method of claim 167, wherein the products of claim 167 are used in (a).

170. The method of claim 163, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

171. A method for evolving a p-nitrophenol phosphonatase from a phosphonatase encoded by a DNA substrate molecule, comprising:

(a) providing library of mutants of the DNA substrate molecule, the library comprising a plasmid expression vector;

(b) transfecting a host, wherein the host phn operon is deleted;

(c) selecting for growth of the transfectants of (b) using a p-nitrophenol phosphonatase as a substrate;

(d) recovering the DNA substrate molecules from transfectants selected from (c); and

(e) recovering a DNA substrate molecule from (d) encoding an evolved phosphonatase.

172. The method of claim 171, wherein (a) - (e) are repeated.

173. The method of claim 171, wherein the phosphonatase is selected from the group consisting of beta-lactamase and alkyl phosphonatase.

174. An evolved p-nitrophenol phosphonatase produced by the method of claim 173.

175. The method of claim 171, wherein the products of (e) are subjected to mutagenesis.

176. The method of claim 175, wherein mutagenesis comprises recursive sequence recombination.

177. The method of claim 175, wherein the products of claim 175 are used in (a).

178. The method of claim 171, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

179. A method for evolving a protease encoded by a DNA substrate molecule comprising:

(a) providing library of mutants of the DNA substrate molecule, the library comprising a plasmid expression vector, wherein the DNA substrate molecule is linked to a secretory leader;

(b) transfecting a host;

(c) selecting for growth of the transfectants of (b) on a complex protein medium; and

(d) recovering a DNA substrate molecule from (c) encoding an evolved protease.

180. The method of claim 179, wherein (a) - (d) are repeated.

181. An evolved subtilisin produced by the method of claim 179.

182. The method of claim 179, wherein the products of (d) are subjected to mutagenesis.

183. The method of claim 182, wherein mutagenesis comprises recursive sequence recombination.

184. The method of claim 182, wherein the products of claim 184 are used in (a).

185. The method of claim 179, wherein the DNA substrate molecule of (d) comprises a library of DNA substrate molecules.

186. The method of claim 179, wherein the protease is a subtilisin.

187. A method for screening a library of protease mutants displayed on a phage to obtain an improved protease, wherein a DNA substrate molecule encoding the protease is fused to DNA encoding a filamentous phage protein to generate a fusion protein, comprising:

(a) providing host cells expressing the fusion protein;

(b) overlaying host cells with a protein net to entrap the phage;

(c) washing the product of (b) to recover phage liberated by digestion of the protein net;

(d) recovering DNA from the product of (c); and

(e) recovering a DNA substrate from (d) encoding an improved protease.

188. The method of claim 187, wherein (a) - (e) are repeated.

189. An evolved protease produced by the method of claim 187.

190. The method of claim 187, wherein the products of (e) are subjected to mutagenesis.

191. The method of claim 190, wherein mutagenesis comprises recursive sequence recombination.

192. The method of claim 190, wherein the products of claim 190 are used in (a).

193. The method of claim 187, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

194. A method for screening a library of protease mutants to obtain an improved protease, the method comprising:

(a) providing a library of peptide substrates, the peptide substrate comprising a fluorophore and a fluorescence quencher;

(b) screening the library of protease mutants for ability to cleave the peptide substrates, wherein fluorescence is measured; and

(c) recovering DNA encoding at least one protease mutant from (b).

195. A method for evolving an alpha interferon gene comprising:

(a) providing a library of mutant alpha interferon genes, the library comprising a filamentous phage vector;

(b) stimulating cells comprising a reporter construct, the reporter construct comprising a reporter gene under control of an interferon responsive promoter, and wherein the reporter gene is GFP;

(c) separating the cells expressing GFP by FACS;

(d) recovering phage from the product of (c); and

(e) recovering an evolved interferon gene from the product of (d).

196. The method of claim 195, wherein the interferon responsive promoter is an MHC I promoter.

197. The method of claim 195, wherein (a) - (e) are repeated.

198. An evolved interferon produced by the method of claim 195.

199. The method of claim 195, wherein the products of (e) are subjected to mutagenesis.

200. The method of claim 199, wherein mutagenesis comprises recursive sequence recombination.

201. The method of claim 199, wherein the products of claim 199 are used in (a).

202. The method of claim 195, wherein the evolved interferon gene of (e) comprises a library of genes.

203. A method for screening a library of mutants of a DNA substrate encoding a protein for an evolved DNA substrate, comprising:

(a) providing a library of mutants, the library comprising an expression vector;

(b) transfecting a mammalian host cell with the library of (a), wherein mutant protein is expressed on the surface of the

cell;

(c) screening or selecting the products of (b) with a ligand for the protein;

(d) recovering DNA encoding mutant protein from the products of (c); and

(e) recovering an evolved DNA substrate from the products of (d).

204. The method of claim 203, wherein the ligand is an antibody.

205. The method of claim 203, wherein the ligand is a substrate and the protein is an enzyme.

206. The method of claim 203, wherein the expression vector comprises an SV40 origin and the host cell is a Cos cell.

207. The method of claim 203, wherein the mutant protein is expressed transiently.

208. The method of claim 203, wherein the host cell further comprises SV40 large T antigen.

209. The method of claim 203, wherein the protein is an antibody.

210. The method of claim 203, wherein (a) - (e) are repeated.

211. The method of claim 203, wherein the DNA substrate molecule encodes all or part of a protein selected from Table I.

212. An evolved protein produced by the method of claim 203.

213. The method of claim 203, wherein the products of (e) are subjected to mutagenesis.

214. The method of claim 213, wherein mutagenesis comprises recursive sequence recombination.

215. The method of claim 213, wherein the products of claim 213 are used in (a).

216. The method of claim 203, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

217. A method for evolving a DNA substrate molecule encoding an interferon alpha, comprising:

(a) providing a library of mutant alpha interferon genes, the library comprising an expression vector wherein the alpha interferon genes are expressed under the control of an inducible promoter;

(b) transfecting host cells with the library of (a);

(c) contacting the product of (b) with a virus;

(d) recovering DNA encoding a mutant alpha interferon from host cells surviving step (c); and

(e) recovering an evolved interferon gene from the product of (d).

218. The method of claim 217, wherein the promoter is a metallothionein promoter.

219. The method of claim 217, wherein the virus is HIV.

220. The method of claim 217, wherein the virus further comprises a conditionally lethal gene.

221. The method of claim 217, wherein the conditionally lethal gene is thymidine kinase.

222. The method of claim 217, wherein the transfected cells are exposed to conditionally lethal selective conditions.

223. The method of claim 217, wherein (a) - (e) are

repeated.

224. An evolved IFNα polymerase produced by the method of claim 217.

225. The method of claim 217, wherein the products of (e) are subjected to mutagenesis.

226. The method of claim 225, wherein mutagenesis comprises recursive sequence recombination.

227. The method of claim 225, wherein the products of claim 218 are used in (a).

228. The method of claim 217, wherein the DNA substrate molecule of (e) comprises a library of DNA substrate molecules.

229. A method for evolving the stability of a protein encoded by a DNA substrate molecule, the DNA substrate molecule comprising a fusion of a DNA sequence encoding the protein with a DNA sequence encoding a filamentous phage protein to generate a fusion protein, the method comprising:

(a) providing a host cell expressing a library of mutants of the fusion protein;

(b) affinity purifying the mutants with a ligand for the protein, wherein the ligand is a human serum protein, tissue specific protein, or receptor;

(c) recovering DNA encoding a mutant protein from the affinity selected mutants of (b); and

(d) recovering an evolved gene encoding the protein from the product of (c).

230. The method of claim 229, wherein the serum protein is serum albumin, immunoglobulin, lipoprotein, haptoglobin, fibrinogen, transferrin, alpha-1 anti-trypsin, or alpha -2 macroglobulin.

231. The method of claim 229, wherein the DNA sequence encoding the filamentous phage protein comprises a phage.

232. The method of claim 229, wherein the DNA sequence encoding the filamentous phage protein comprises a phagemid.

233. The method of claim 229, wherein the products of step (a) are derivitized with a half-life extending moiety.

234. The method of claim 229, wherein the moiety is polyethylene glycol.

235. The method of claim 229, wherein the DNA substrate molecule comprises a fusion of nucleic acid encoding the protein with nucleic acid encoding an epitope tag.

236. The method of claim 235, wherein the products of (a) are contacted with a protease prior to (b).

237. The method of claim 235, wherein the ligand is an antibody specific for the epitope tag.

238. The method of claim 229, wherein the protein is selected from Table I.

239. The method of claim 229, wherein the products of (a) are subjected to heat, metal ions, non-physiological pH, lyophilization, or freeze-thawing before (b).

240. The method of claim 229, wherein (a) - (e) are repeated.

241. An evolved polymerase produced by the method of claim 229.

242. The method of claim 229, wherein the products of (d) are subjected to mutagenesis.

243. The method of claim 242, wherein mutagenesis comprises recursive sequence recombination.

244. The method of claim 242, wherein the products of claim 242 are used in (a).

245. The method of claim 229, wherein the evolved gene of (d) comprises a library of DNA substrate molecules.

246. A method for evolving a protein having at least two subunits, comprising:

(a) providing a library of mutant DNA substrate molecules for each subunit;

(b) recombining the libraries into a library of single chain constructs of the protein, the single chain construct comprising a DNA substrate molecule encoding each subunit sequence, the subunit sequence being linked by a linker at a nucleic acid sequence encoding the amino terminus of one subunit to a nucleic acid sequence encoding the carboxy terminus of a second subunit;

(c) screening or selecting the products of (b),

(d) recovering recombinant single chain construct DNA substrate molecules from the products of (c);

(e) subjecting the products of (d) to mutagenesis; and

(f) recovering an evolved single chain construct DNA substrate molecule from (e).

247. The method of claim 246, wherein the products of (b) are displayed on a phage.

248. The method of claim 246, wherein the protein is selected from Table I.

249. The method of claim 246, wherein (a) - (f) are repeated.

250. An evolved protein produced by the method of claim

246.

251. The method of claim 246, wherein the products of (f) are subjected to mutagenesis.

252. The method of claim 246, wherein mutagenesis comprises recursive sequence recombination.

253. The method of claim 246, wherein the products of claim 246 are used in (a).

254. The method of claim 246, wherein the evolved DNA substrate molecule of (f) comprises a library of DNA substrate molecules.

255. A method for evolving the coupling of a mammalian 7-transmembrane receptor to a yeast signal transduction pathway, comprising:

(a) expressing a library of mammalian G alpha protein mutants in a host yeast cell, wherein the host cell expresses the mammalian 7-transmembrane receptor and a reporter gene, the receptor gene geing expressed under control of a yeast pheromone responsive promoter;

(b) screening or selecting the products of (a) for expression of the reporter gene in the presence of a ligand for the 7-transmembrance receptor; and

(c) recovering DNA encoding an evolved G alpha protein mutant from screened or selected products of (b).

256. The method of claim 255, wherein the products of (c) are subjected to mutagenesis.

257. The method of claim 256, wherein mutagenesis comprises recursive sequence recombination.

258. The method of claim 255, wherein the products of claim 255 are used in (a).

259. The method of claim 255, wherein (a) - (c) are repeated.

260. An evolved G alpha protein produced by the method of claim 255.

261. The method of claim 255, wherein the reporter gene is luciferase.

262. The method of claim 255, wherein the pheromone responsive promoter is positively regulated by GAL4 and wherein GAL4 is expressed under the control of a pheromone sensitive, GAL4 enhanced promoter.

263. A method for recombining at least a first and second DNA substrate molecule, comprising:

(a) transfecting a host cell with at least a first and second DNA substrate molecule wherein the at least a first and second DNA substrate molecules are recombined in the host cell;

(b) screening or selecting the products of (a) for a desired property; and

(c) recovering recombinant DNA substrate molecules from (b).

264. The method of claim 263, wherein the products of (c) are subjected to mutagenesis.

265. The method of claim 264, wherein the mutagenesis comprises recursive sequence recombination.

266. The method of claim 263, wherein (a) - (c) are repeated.

267. The method of claim 263, wherein the products of claim 263 are used in (a).

268. A method for evolving a DNA substrate sequence

encoding a protein of interest, wherein the DNA substrate comprises a vector, the vector comprising single-stranded DNA, the method comprising:

(a) providing single-stranded vector DNA and a library of mutants of the DNA substrate sequence;

(b) annealing denatured double-stranded DNA from the library of (a) to the single stranded vector DNA of (a);

(c) transforming the products of (b) into a host;

(d) screening the product of (c) for a desired property; and

(e) recovering evolved DNA substrate DNA from the products of (d).

269. The method of claim 268, wherein the product of (e) is subjected to mutagenesis.

270. The method of claim 269, wherein mutagenesis comprises recursive sequence recombination.

271. The method of claim 269, wherein the product of claim 269 is used in (a).

272. The method of claim 268, wherein the host is a mutS host.

273. The method of claim 268, wherein the vector is a phagemid.

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